

Psychopharmacology (2004) 175: 7–17  
DOI 10.1007/s00213-004-1782-1

ORIGINAL INVESTIGATION

Frank Boeckler · Holger Russig · Weining Zhang ·  
Stefan Löber · John Schetz · Harald Hübner ·  
Boris Ferger · Peter Gmeiner · Joram Feldon

## FAUC 213, a highly selective dopamine D<sub>4</sub> receptor full antagonist, exhibits atypical antipsychotic properties in behavioural and neurochemical models of schizophrenia

Received: 5 February 2003 / Accepted: 10 November 2003 / Published online: 6 March 2004  
© Springer-Verlag 2004

**Abstract** *Rationale:* 2-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-a]pyridine (FAUC 213) is a highly selective antagonist at the dopamine D<sub>4</sub> receptor subtype. It was designed as a derivative of two partial antagonists and has been proven to be a complete antagonist in mitogenesis assay. *Objectives:* In the present study, FAUC 213 was examined for antipsychotic properties in animal models of behavioural neurobiology and neurochemistry. *Methods:* Different concentrations of FAUC 213 were screened for effects on spontaneous, as well as amphetamine-induced, locomotor activity and apomorphine-induced prepulse disruption. The liability of causing extrapyramidal side effects was investigated in models of catalepsy and by high-performance liquid chromatography (HPLC) detection of dopamine turnover in several brain regions. The application schedule was validated, and the bioavailability of the compound determined, by means of a HPLC-pharmacokinetic study. *Results:* A significant effect in both the reduction of amphetamine-induced locomotor hyperactivity and the restoration of apomorphine-disrupted prepulse inhibition was found at 30 mg/kg. This dose proved not to be high enough to induce catalepsy or to increase dopamine turnover in the dorsal striatum, nucleus accumbens and medial prefrontal cortex.

The selective D<sub>4</sub> antagonist FAUC 213, therefore, is not believed to mediate the above-mentioned effects via D<sub>2</sub> receptor antagonism, but a partial involvement of 5-HT<sub>2</sub>- and  $\alpha_1$ -receptors cannot be ruled out at present. *Conclusions:* We have gathered evidence that FAUC 213 exhibits atypical antipsychotic characteristics.

**Keywords** Dopamine D<sub>4</sub> selective · FAUC 213 · Complete antagonist · Prepulse inhibition · Locomotor activity · Catalepsy · Dopamine turnover · Atypical antipsychotic

### Introduction

Since the dopamine D<sub>4</sub> receptor was identified in 1990 by Van Tol et al., there has been increasing interest in this receptor subtype as a putative target for the treatment of schizophrenia. The D<sub>4</sub> receptor belongs to the D<sub>2</sub>-like receptor family (D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>) that is characterised by structural and functional similarities, such as a certain degree of homology and congeneric G-protein coupling. As the clinical potencies of typical neuroleptics, like haloperidol, seem to be intimately correlated with their D<sub>2</sub> receptor affinities (reviewed by Snyder 1996), the fact that some of these drugs also exhibit remarkable binding properties towards the other D<sub>2</sub>-like receptors also aroused fundamental interest in the D<sub>3</sub> and D<sub>4</sub> receptor subtype. Moreover, the considerable pharmacological profile of the atypical antipsychotic clozapine has focused special attention on the D<sub>4</sub> receptor, since it shows a moderate 2-fold to 5-fold preference for D<sub>4</sub> over D<sub>2</sub> (Chabert et al. 1994; Roth et al. 1995; Newman-Tancredi et al. 1997; Löber et al. 2001) and good correlations between the clinically efficacious dose and its D<sub>4</sub> affinity (Seeman 1995; Seeman and Van Tol 1995). It also shows superior efficacy in refractory schizophrenia and negative symptoms such as amotivation and social withdrawal, as well as a minimal risk or even absence of side effects such as the extrapyramidal symptoms (EPS) and elevation of the prolactin level (reviewed by Remington and Kapur 2000).

F. Boeckler · H. Russig · W. Zhang · B. Ferger · J. Feldon (✉)  
Laboratory of Behavioural Neurobiology, Swiss Federal  
Institute of Technology Zürich,  
Schorenstrasse 16,  
8603 Schwerzenbach, Switzerland  
e-mail: feldon@behav.biol.ethz.ch  
Tel.: +41-1-6557448  
Fax: +41-1-6557203

F. Boeckler · S. Löber · H. Hübner · P. Gmeiner  
Department of Medicinal Chemistry, Emil Fischer Center,  
Friedrich-Alexander University,  
Schuhstrasse 19,  
91052 Erlangen, Germany

J. Schetz  
Department of Pharmacology & Neuroscience, University of  
North Texas Health Science Center,  
Fort Worth, TX 76107, USA

The clinical use of clozapine is unfortunately restrained by the approximate 1% incidence rate of agranulocytosis, a presumably immune-mediated fatal lack of white blood cells. The challenge is, therefore, to develop novel atypical antipsychotics mimicking the unusual properties of clozapine. Due to the binding profile of clozapine, there are several approaches reflecting the different views about the receptor basis of this atypical drug action (reviewed by Seeman et al. 1997). These various hypotheses include low affinity for D<sub>2</sub> as exclusive drug action, concurrent antagonism at D<sub>2</sub> and muscarinergic receptors, balanced blocking of D<sub>2</sub> and 5-HT<sub>2A</sub> receptors, and selective antagonism at D<sub>4</sub> receptors. With respect to the D<sub>4</sub> research, numerous investigations have focused on receptor distribution in different brain regions, involving autoradiographic labelling of receptor binding sites (Primus et al. 1997; Lahti et al. 1998; De la Garza and Madras 2000), neuroanatomical detection of receptor protein using specific antibodies (Mrzljak et al. 1996; Ariano et al. 1997; Defagot et al. 1997) and localisation of D<sub>4</sub>-mRNA by means of Northern blot/reverse-transcription polymerase chain reaction (RT-PCR) (Van Tol et al. 1991; Matsumoto et al. 1995, 1996; Mulcrone and Kerwin 1997). These studies suggest, in a largely consistent manner, that D<sub>4</sub> receptors are preferentially located in limbic or cortical structures such as the amygdala and hippocampus or the prefrontal cortex, respectively. These are known to be involved in the regulation of mood and cognition and are suspected of playing a part in the aetiology of schizophrenia, although it remains controversial whether D<sub>4</sub> levels are increased in schizophrenic brains (Seeman et al. 1993, 1995) or not (Reynolds and Mason 1995). Also, lower D<sub>4</sub> levels were found, by comparison, in the striatum and other regions modulating motor functions, suggesting a small probability of causing EPS. Taken together, this evidence indicated that selective D<sub>4</sub> antagonists could be valuable in further investigations of the relevance of this receptor subtype in the treatment of schizophrenia. Now, after several of these compounds have been tested and published, the issue remains controversial (Bristow et al. 1997; Patel et al. 1997; Mansbach et al. 1998). For instance U-101958, a prominent D<sub>4</sub> selective antagonist, was found to exhibit some agonistic activity (Gazi et al. 1998, 1999). In contrast, 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-a]pyridine (FAUC 213) was shown to be not only highly D<sub>4</sub> selective (Table 1), but also a complete antagonist with less than 3% agonistic activity compared with the full agonist quinpirole (Löber et al. 2001). FAUC 213 was therefore considered worthy of having its biological profile examined with methods of behavioural neurobiology and neurochemistry as described in this article.

**Table 1** Receptor binding data of FAUC 213 and FAUC 113 compared with clozapine at human dopamine, porcine serotonin and  $\alpha_1$  receptors according to Löber et al. (2001). K<sub>i</sub> values are the means of two to four independent experiments  $\pm$ SEM using eight different concentrations, each in triplicate

Compound	K <sub>i</sub> [nM] $\pm$ SEM	<sup>3</sup> [H]Spiperone				<sup>3</sup> [H] $\beta$ -HO-DPAT		<sup>3</sup> [H]Ketanserin	<sup>3</sup> [H]Prazosin	Receptor selectivity		
		hD <sub>2</sub> (long)	hD <sub>2</sub> (short)	hD <sub>3</sub>	hD <sub>4,4</sub>	p5-HT <sub>1A</sub>	p5-HT <sub>2</sub>			hD <sub>2</sub> (short)/hD <sub>4,4</sub>	p5-HT <sub>2</sub> /hD <sub>4,4</sub>	p $\alpha_1$ /hD <sub>4,4</sub>
FAUC 213	3400 $\pm$ 450	6300 $\pm$ 1900	5300 $\pm$ 720	2.2 $\pm$ 0.23	1200 $\pm$ 100	520 $\pm$ 80	270 $\pm$ 20	2900	240	120		
FAUC 113	3200 $\pm$ 400	4300 $\pm$ 650	5000 $\pm$ 650	3.6 $\pm$ 0.87	1400 $\pm$ 130	380 $\pm$ 140	240 $\pm$ 10	1200	110	67		
Clozapine	41 $\pm$ 1.5	28 $\pm$ 0.50	960 $\pm$ 45	16 $\pm$ 0.50	460 $\pm$ 120	12 $\pm$ 1.0	n.d.	1.8	0.8	n.d.		

## Experimental procedures

### Animals

A total of 94 male adult Wistar rats (Zur:Wist[HanIbm]; Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing 300–350 g at the time of the experiments, were used in this study. The animals were housed in groups of four per cage under a reversed light-dark cycle (lights on: 1900–0700 hours) in a temperature- ( $21 \pm 1^\circ\text{C}$ ) and humidity- ( $55 \pm 5\%$ ) controlled room with free access to food (Kliba 3430, Klibamühlen, Kaiseraugst, Switzerland) and water. Beginning 3 days before the experiments, and continuing throughout the study, all rats were handled daily. Behavioural testing was carried out during the dark phase of the cycle. All experiments were conducted in accordance with Swiss regulations for animal experimentation.

### Drugs

All drug solutions were freshly prepared on the day of experiment. FAUC 213 was synthesised at the Department of Medicinal Chemistry of the Friedrich-Alexander University Erlangen-Nürnberg and prepared for peroral application as a 7.5, 15 and 30-mg/ml solution in PEG400/200 mM acetate-buffer ( $\text{pH} \sim 3.9$ ) = 40/60 with the final pH adjusted to 4.3. D-Amphetamine sulfate (Sigma Chemical Company, St. Louis, USA) was dissolved in a 0.9% NaCl solution for intra-peritoneal injection in order to obtain a concentration of 1.0 mg/ml calculated as the salt. For increased stability, R(-)-apomorphine hydrochloride (Sigma Chemical Company) was dissolved in 0.9% NaCl containing 0.1% ascorbic acid giving a 0.1-mg/ml solution for subcutaneous administration. Haloperidol was diluted from ampoules with 5-mg/ml solvent (Janssen-Cilag, Baar, Switzerland) with 0.9% saline to a concentration of 0.3 mg/ml and adjusted to pH 5.5 by addition of  $\text{Na}_2\text{CO}_3$  to enable intra-peritoneal injection.

### Apparatus and procedures for behavioural testing

#### *Detection of locomotor activity*

Locomotor activity was measured in 15 stations, each being a clear plastic compartment 25 cm wide  $\times$  40 cm long  $\times$  40 cm high and completely embedded within an individual sound-attenuating wooden cabinet. One end wall of each compartment was made of wood, and the floor consisted of a black removable pan containing a thin layer of dark absorbent, autoclaved earth. A large drop-down door in the front wall of every compartment allowed easy access to the animal. A fan mounted on the wall of each cabinet provided adequate ventilation and a camera was mounted in the ceiling, centred approximately 49 cm above the compartment floor. The field of vision of this camera included the entire area in which the animal was freely able to move. The stations were located in a well-ventilated, temperature-, humidity- and sound-controlled room used only for this experiment. All the cameras were connected to a 16-channel multiplexer (Sony model YS-DX216CE) located in an adjacent room, and the multiplexer was connected in turn to a Dell OptiPlex GXpro computer running image-analysis software (Visual Basic program based on an NIH Image Analysis script). Each white Wistar rat was monitored against a darker background. An image from each of the stations was taken every second and compared with the previous one. The activity or animal's displacement was measured as pixel changes (from light to dark and vice versa) per second. One-second activity values ranged from 0% (no movement) to approximately 7.5%. The multiplexed images from the 15 stations could be recorded simultaneously on one videotape with a Sony SVT-1000P video recorder and viewed on a single monitor.

### *Prepulse inhibition*

Prepulse inhibition (PPI) testing was conducted in four ventilated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), each containing a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a Plexiglas frame. Acoustic pulses and prepulses were presented via a speaker mounted 24 cm above the tube. Motion inside the tube was detected by a piezoelectric accelerometer below the frame. The amplitude of the whole body startle to an acoustic pulse was defined as the average of 100 1-ms accelerometer readings collected from pulse onset. Delivery of the acoustic stimuli and recording of startle responses were controlled by a computer. The animals were tested in squads of four with startle chambers counterbalanced across the different experimental groups. After placing the animals inside the tubes, the startle session began with a 5-min acclimatisation period during which only a 68-dB(A) background noise was presented. This background noise continued throughout the session. After the acclimatisation period, four startle pulses of 120 dB(A), each of 30 ms duration, were presented. These served to achieve a relatively stable level of startle reactivity for the remainder of the test session since the most pronounced habituation of the startle reflex occurs within the first few startle-pulse presentations (Geyer et al. 1990; Koch 1999). To measure PPI, six blocks of 11 trials each were presented. The 11 trials in each block included: two "pulse-alone" trials, one "prepulse followed by pulse" trial for each of four prepulse intensities, one "prepulse alone" trial for each of four prepulse intensities, and one "no stimulus" trial. Prepulses were broad-band bursts of 20 ms duration and intensity of either 72, 76, 80, or 84 dB(A). The time interval between prepulse offset and pulse onset was always 80 ms. The different trial types were presented pseudo-randomly with an intertrial interval of 10–20 s (average 15 s). Altogether, a complete test session lasted about 23 min.

### *Catalepsy bar test*

The animals were tested in a Perspex box measuring 26 $\times$ 17 $\times$ 17 cm containing a bar mounted 10 cm above the floor. Following a habituation period of 1 min, the animals were gently placed with their front paws on the bar while their hind paws remained on the floor. The latency for the rat to remove both front paws from the bar was measured, up to a maximum of 120 s.

### *Catalepsy paw test*

A 30 $\times$ 30-cm Perspex plate mounted on plastic feet 20 cm above the floor was utilised. The plate contained two holes of 4 cm diameter for the forelimbs and two holes of 5 cm diameter for the hindlimbs, as well as an opening for the tail. The rat was held behind the forelimbs and gently placed in the holes, first with the hindlimbs and then with the forelimbs. The latency for the rat to retract one of the front paws was measured from the moment the rat retracted one of the hind paws (up to a maximum of 120 s).

### Experimental design

#### *Locomotor activity*

Spontaneous and amphetamine-induced locomotion was assessed in the 15 test boxes with 30 rats on two successive days. On day 1, each rat was placed in one of the boxes and left there for 60 min to measure spontaneous (baseline) activity. The rats were then allocated to five treatment groups (VEH+VEH, VEH+AMPH, F7.5+AMPH, F15+AMPH and F30+AMPH) consisting of  $n=6$  animals per group matched for baseline locomotor activity. On day 2 (treatment day), each rat was perorally pretreated with FAUC 213 in three different drug doses (7.5, 15 and 30 mg/kg bodyweight) or

vehicle (made-up of the identical solute as used to dissolve FAUC 213: PEG400/100mM-acetate-buffer(pH~3.9)=40:60 adjusted to a pH~4.3), and then immediately placed in the same box as the previous day for 60 min to monitor the influence of FAUC 213 versus VEH on spontaneous locomotion. Following this, all rats were injected intra-peritoneally with 1.0 mg/kg amphetamine or 0.9% saline and instantly replaced in their boxes for another 105 min to monitor the effects of FAUC 213 pretreatment versus VEH on AMPH-induced hyperactivity.

### PPI experiments

Thirty-nine naive rats were used for the PPI experiment, conducted on two successive days. On day 1, all rats were subjected to baseline PPI testing and were then matched according to their PPI and startle response in four treatment groups. On day 2, the effect of pretreatment with FAUC 213 at dose levels of 15 mg/kg and 30 mg/kg versus VEH pretreatment was tested on apomorphine-disrupted PPI. Pre-treatment was applied perorally 45 min prior to the subcutaneous injection of 0.1 mg/kg APO versus VEH (0.1% ascorbic acid in 0.9% saline). Since APO penetrates quickly into the brain, the animals were placed in the PPI-boxes approximately 5 min after the injection. The four differently treated groups had sizes as follows: VEH+APO:  $n=9$ ; VEH+VEH, F15+APO and F30+APO:  $n=10$ .

### Catalepsy experiments

The experimental protocol consisted of three groups of eight animals. Haloperidol (0.3 mg/kg, i.p.), FAUC 213 (30 mg/kg, p.o.) or vehicle (PEG400/100mM-acetate-buffer=40:60-adj. to pH~4.3, p.o.) was applied 45 min before testing. The rats were first monitored in the catalepsy bar test and then in the catalepsy paw test, as previously described.

### Neurochemical and pharmacokinetic experiments

#### Postmortem HPLC analysis of dopamine metabolism

After the catalepsy testing procedure, the animals were taken to a separate room where they were sacrificed by decapitation exactly 60 min after the application of FAUC/HAL/VEH. The brains were removed rapidly, quickly frozen and stored at  $-20^{\circ}\text{C}$  until preparation.

### Tissue preparation

The frozen brains were placed ventral side up in a rat brain matrix (Harvard Apparatus, South Natick, MA, USA) on an ice-chilled plate. Double-edge blades were used to prepare coronal sections approximately 2 mm thick. The partially frozen slices were placed on an ice-cold dissection plate for the removal of discrete brain regions using a 2-mm diameter punch tip together with the MP-600 micropunch system (Harvard Apparatus). Tissue punches from the right and left hemispheres were taken together for subsequent neurochemical analysis. Three structures were prepared according to the atlas of Paxinos and Watson (1986): the medial prefrontal cortex (mPFC) was prepared from the first slice (Bregma 4.80 to 2.80), the nucleus accumbens (NAcc) from the next slice (Bregma 2.80 to 0.80), and the dorsal striatum (dStr) from the adjacent slice (Bregma 0.80 to  $-1.20$ ). The punching was accomplished as shown in Fig. 1A for the mPFC, 1B for the NAcc and 1C for the dStr. The tissue samples were weighed and placed in 1.5-ml plastic tubes containing ice-cooled perchloric acid (300  $\mu\text{l}$ , 0.4 M), homogenised for 10 s using ultrasound and centrifuged for 20 min at 15000 g and  $4^{\circ}\text{C}$ . The supernatant was passed through a 0.2- $\mu\text{m}$  filter and kept at  $4^{\circ}\text{C}$  until high-performance liquid chromatography (HPLC) analysis.

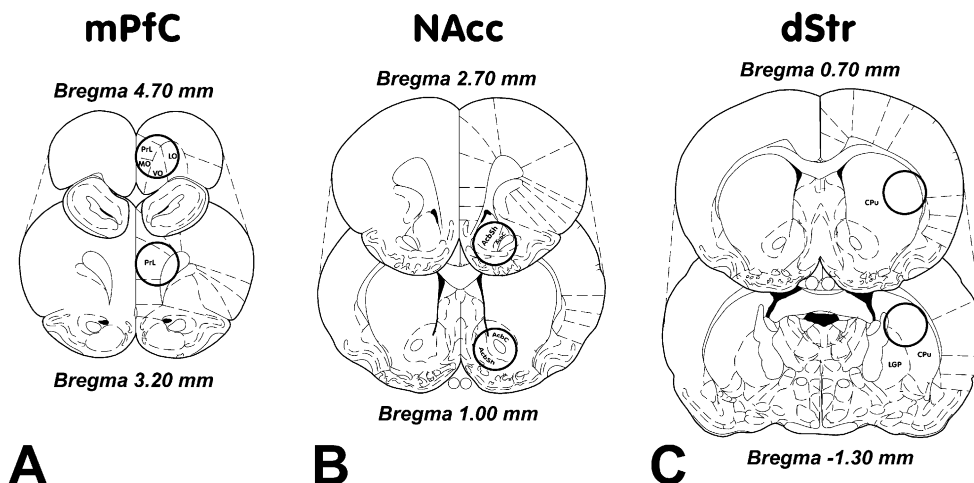
### Determination of dopamine and metabolites

Dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were analysed using reversed-phase ion-pair chromatography combined with electrochemical detection under isocratic conditions. The detector potential was set at +750 mV using a glassy carbon electrode and a Ag/AgCl reference electrode. The mobile phase (34.88 mM citric acid, 54.37 mM sodium acetate, 0.67 mM ethylene diamine tetraacetic acid disodium salt, 0.69 mM 1-octanesulfonic acid, 4% methanol in HPLC grade water, final pH 4.10) was delivered at a flow rate of 1.0 ml/min at  $30^{\circ}\text{C}$  onto the reversed-phase column [100 $\times$ 3.0 mm with guard column 10 $\times$ 3.0 mm filled with Chromsher 5B (Varian ChromSep, Palo Alto, USA)]. Aliquots (20  $\mu\text{l}$ ) were injected by an autosampler with cooling module set at  $4^{\circ}\text{C}$ . Data were calculated using an external standard calibration.

### Pharmacokinetic study

Twenty male Wistar rats were treated with FAUC 213 (30 mg/kg, p.o.) 30, 45, 60, 90 or 120 min before being sacrificed by decapitation. Four untreated animals were also decapitated to enable calibration of the extraction process by spiking and extracting comparable brain

**Fig. 1** Silhouette drawings of micropunchings onto representative sections of the rat brain. Localisation of the micropunchings for the medial prefrontal cortex (mPFC) (A), the nucleus accumbens (NAcc) (B), and the dorsal striatum (dStr) (C) are depicted by black circles 2 mm in diameter. The three tissue slice preparations are represented by one layer drawing close to the upper border and one close to the lower border, chosen according to Paxinos and Watson (1986): slice A (4.80–2.80 mm bregma), slice B (2.80–0.80 mm bregma), slice C (0.80 mm to  $-1.20$  mm bregma)





tissue under analogous conditions. All 24 brains were rapidly removed, quickly frozen and stored at  $-80^{\circ}\text{C}$  until further analysis.

### Sample preparation

The frozen brains were weighed before being placed in 50-ml plastic tubes and covered with 15 ml chloroform. The brain tissue was homogenised in a cooling room at  $4^{\circ}\text{C}$  using an Ultra-Turrax T25 (Janke&Kunkel IKA-Labortechnik, Staufen i. Br.) at  $\sim 20000$  rpm for 60 s and stored afterwards on ice for 120 s. A 5-N NaOH-solution (200  $\mu\text{l}$ ) was then added, and this mixture was homogenised two further times for 60 s each, storing it for another 60 s on ice in-between. An additional 15 ml chloroform was used to wash the adherent homogenate from the ultraturax into the tube. These were stored on ice until re-mixing the tubes for 1 min by shaking and then centrifuging at 3000 rpm for 15 min. The organic layer was subsequently transferred into a round glass flask and evaporated to dryness at 474 mbar and  $40^{\circ}\text{C}$ . The residue was redissolved and sonicated for 15 min in 3 ml chloroform, passed through a  $0.2\text{-}\mu\text{m}$  filter and evaporated under a stream of nitrogen at  $70^{\circ}\text{C}$ . Finally, it was redissolved again by a 15-min ultrasound treatment in 500  $\mu\text{l}$  of a 60:40 (v/v) mixture of acetonitrile-water (containing 0.1% formic acid) and filtered through a membrane filter and stored at  $-80^{\circ}\text{C}$  until HPLC analysis.

### Detection of FAUC 213 brain levels

FAUC 213 was analysed using reversed-phase ion-pair chromatography combined with UV detection at 258-nm wave length under gradient-elution conditions. The mobile phase (mph1: acetonitrile/mph2:25 mM  $\text{KH}_2\text{PO}_4$ , 5 mM pentanesulfonic acid in HPLC grade water, final pH 7.00) was delivered at a flow rate of 1.0 ml/min at  $40^{\circ}\text{C}$  onto the reversed-phase column [250 $\times$ 3.0 mm with guard column 40 $\times$ 3.0 mm filled with LiChrosorb RP18 (Merck KG, Darmstadt, Germany)] of a particle size of 7  $\mu\text{m}$  or 10  $\mu\text{m}$ , respectively. The composition of the mobile phase was controlled by a gradient-time program consisting of five time periods: 0–15 min: plateau at 60% mph1/15–20 min: ramp from 60% to 90% mph1/20–30 min: plateau at 90% mph1/30–40 min: ramp from 90% to 60% mph1/40–60 min: plateau at 60% mph1 (to restore starting conditions). Fifty microliter aliquots were injected. Data were

calculated by an external standard calibration. Brain tissue of untreated animals was spiked with different concentrations of FAUC 213 in the range of 500 ng to 6000 ng per sample.

### Data analysis

All statistical analyses were performed with the StatView software system (Abacus Concepts, Inc., Berkeley, CA, 1992) using analysis of variance (ANOVA). Data are presented as means. In the text and in bar plots, the variability is indicated by the standard error of the mean (SEM). Fisher's protected least significant difference test was used for post-hoc comparisons following a significant main effect or interaction revealed by ANOVA. Significant differences were accepted at  $P < 0.05$ .

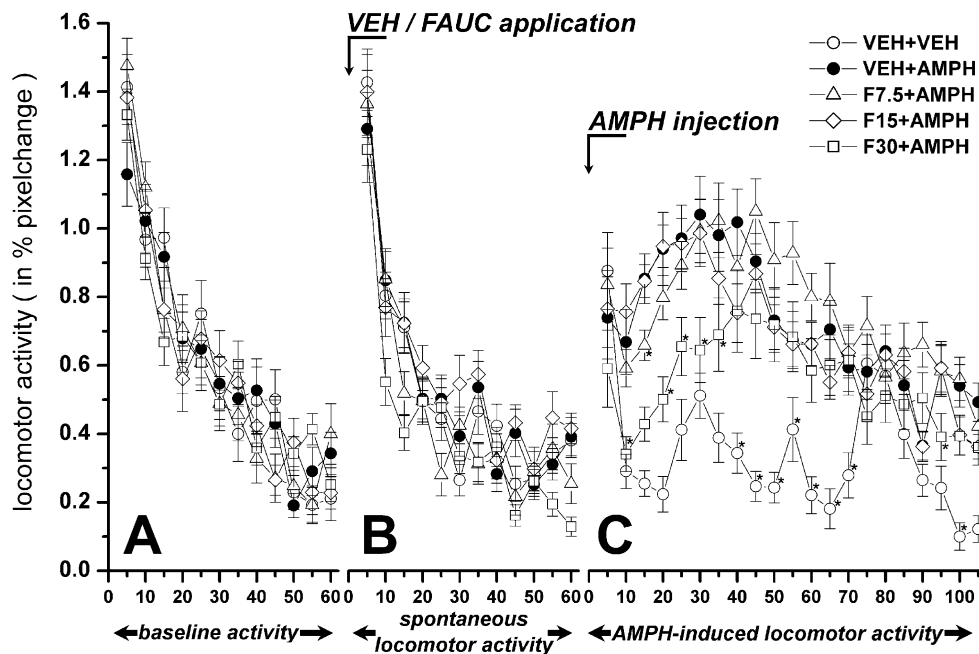
### Locomotor activity experiment

The locomotor activity of the rat was calculated as mean percentage pixel-change for each 5-min block of testing. A  $5 \times 12$  ANOVA consisting of a between-subjects factor of treatment (VEH+VEH, VEH+AMPH, F7.5+AMPH, F15+AMPH, F30+AMPH) and a repeated-measurements factor of 12 5-min blocks was used to analyse the 1-h baseline activity and also the 1-h activity following injection of vehicle or FAUC 213 at doses of 7.5, 15 and 30 mg/kg. A similar  $5 \times 21$  ANOVA was used to examine the 105 min following AMPH injection.

### PPI experiment

The percentage of PPI (%PPI) induced by each prepulse intensity was calculated as:  $[100 - (100 \times \text{startle amplitude on prepulse followed by pulse-trial}) / (\text{startle amplitude on pulse-alone trial})]$  from the data taken during the six test blocks to measure PPI. The overall mean %PPI was calculated for the four prepulse intensities, whereas the mean startle amplitude was calculated as the average response to the 16 "pulse-alone" trials. The analyses consisted of three-way ANOVAs with a between-subjects factor of treatment (VEH+VEH, VEH+APO, F15+APO, F30+APO) and two within-subjects factors of day (baseline day, treatment day) and either the

**Fig. 2** Spontaneous and amphetamine-induced locomotor activity in the open field after peroral application of different doses of FAUC 213. Baseline activity (A) was recorded for 60 min 1 day before treatment. Spontaneous locomotor activity (B) was measured for 60 min following peroral application of FAUC 213 at doses of 7.5, 15 and 30 mg/kg or vehicle ( $n=6$  rats per group). Amphetamine (1.0 mg/kg i.p.) was then injected and the induced hyperactivity was recorded for 105 min (C). All data are given as mean values of 5-min bins  $\pm$  SEM. Asterisks denote only the minimum effective dose compared with vehicle/amphetamine-treated animals ( $*P < 0.05$ )



16 “pulse-alone” presentations (startle responses) or the four prepulse intensities (%PPI).

#### Catalepsy bar and paw test

The cataleptic behaviour of the rat was scored from 1 to 5 according to the (square root transformed) retraction times (min): 1= $t < 0.09$ , 2= $t < 0.36$ , 3= $t < 0.81$ , 4= $t < 1.44$ , 5= $t \geq 1.44$  min. ANOVAs consisting of the between-subjects factor of treatment (VEH, FAUC 213, HAL) were used for the bar test as well as the FRT (forelimb retraction time) and HRT (hindlimb retraction time) of the paw test.

#### Postmortem HPLC-analysis of dopamine metabolism

Dopamine turnover was calculated as ratio of the concentrations (ng/mg tissue) of (HVA + DOPAC)/DA for the separately analysed samples of the three different brain regions: NAcc and dStr. A 3×3 ANOVA with a between-subjects factor of treatment (VEH, FAUC 213, HAL) and a within-subjects factor of region (NAcc and dStr) was performed to evaluate the effects of FAUC 213 versus haloperidol and vehicle on dopamine metabolism.

## Results

#### Activity during the 60 min on the baseline day

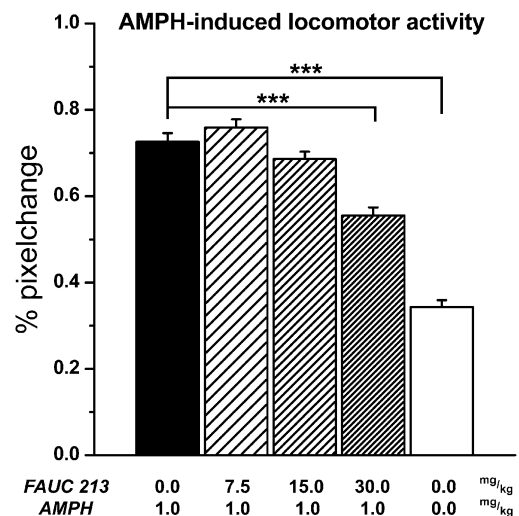
Baseline locomotor activity decreased over the 12 5-min bins ( $F_{11,275}=72.93$ ,  $P<0.001$ ), with no significant difference detected between the treatments. This outcome reflected habituation to the apparatus (Fig. 2A).

#### Activity during the 60-min monitoring spontaneous locomotion after FAUC/VEH application

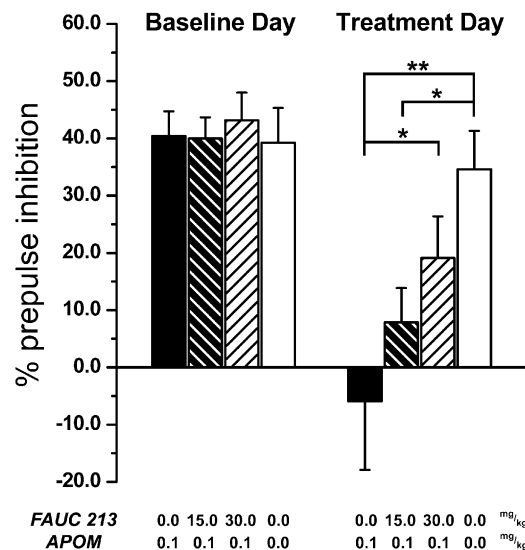
Locomotor activity decreased over the 12 5-min bins ( $F_{11,275}=75.18$ ,  $P<0.001$ ), again reflecting habituation to the apparatus. No significant differences between treatments were found, indicating that spontaneous locomotor activity is not affected by the application of FAUC 213 alone (Fig. 2B).

#### Activity during the 105-min monitoring locomotor hyperactivity following amphetamine injections

The analysis of amphetamine-induced locomotor hyperactivity yielded a significant main effect of treatment ( $F_{4,25}=2.78$ ,  $P<0.05$ ), a significant effect of 5-min bins ( $F_{20,500}=8.24$ ,  $P<0.001$ ) and a significant effect of treatment × 5-min bin interaction ( $F_{80,500}=1.33$ ,  $P<0.001$ ). Post-hoc tests revealed that, compared with VEH injection, all four amphetamine-injected groups showed a significant elevation in activity (all  $P<0.001$ ). Only pretreatment with FAUC 30 mg/kg was able to significantly reduce this elevation in AMPH-induced locomotor hyperactivity ( $P<0.001$ ) (Fig. 3C and Fig. 4).



**Fig. 3** Inhibition of amphetamine-induced locomotor activity in rats. Bar plots are calculated as the mean values of 5-min bins as shown in C ( $\pm$ SEM) of  $n=6$  rats per group. Asterisks denote the significant differences compared with vehicle/amphetamine-treated animals (\*\* $P<0.001$ )



**Fig. 4** Effects of pretreatment with FAUC 213 (15 mg/kg or 30 mg/kg, p.o.) on prepulse inhibition disruption by apomorphine (0.1 mg/kg, s.c.) in Wistar rats. Pretreatment was applied 45 min prior to apomorphine. Values are represented as means $\pm$ SEM of  $n=9-10$  rats per group. Asterisks denote significant differences between treatments as revealed by Fisher's PLSD post-hoc tests (\*\* $P<0.01$ , \* $P<0.05$ )

#### Effects of apomorphine and apomorphine + FAUC 213 (15 mg/kg and 30 mg/kg) on startle response

There was no significant difference in the startle response amplitude of the animals between the different treatment groups. A habituation of the startle response amplitude over the 16 “pulse-alone” presentations was apparent ( $F_{15,525}=19.76$ ,  $P<0.001$ ). In addition, the significant effect of day ( $F_{1,35}=11.09$ ,  $P<0.0025$ ) and the interaction of day × pulse presentation ( $F_{15,525}=2.52$ ,  $P<0.002$ )

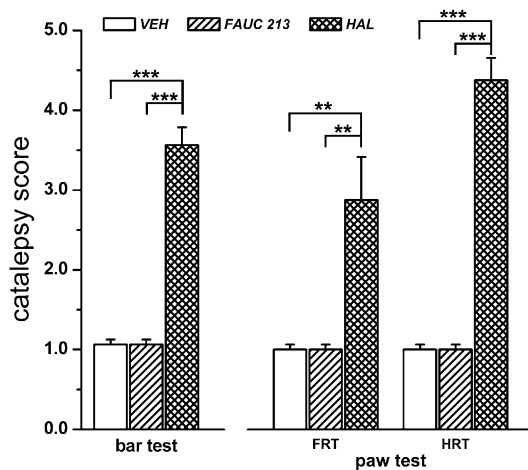
reflected the steeper habituation on day 2 compared with day 1 (data not shown).

#### Effects of apomorphine and apomorphine + FAUC 213 (15 mg/kg and 30 mg/kg) on PPI

The analysis of PPI yielded a nearly significant main effect of treatment ( $F_{3,35}=2.65$ ,  $P<0.065$ ), a significant main effect of day ( $F_{1,35}=40.95$ ,  $P<0.001$ ) and a significant interaction of treatment  $\times$  day ( $F_{3,35}=4.25$ ,  $P<0.015$ ). This reflected the fact, that while apomorphine in its own right completely abolished the PPI response, FAUC 213 dose dependently reduced this disruption (Fig. 4). Post-hoc comparisons revealed that on the treatment day (day 2) only FAUC 30 mg/kg was able to significantly restore the PPI reduction caused by the APO treatment ( $P<0.04$ ), to the extent that this group did not differ significantly from the VEH group ( $P>0.16$ ). A significant effect of prepulse intensity ( $F_{4,105}=29.02$ ,  $P<0.001$ ) reflected the increased effectiveness of higher intensities of the prepulse stimulus to induce stronger PPI.

#### Effects of FAUC 213 (30 mg/kg) vs haloperidol (0.3 mg/kg) and vehicle on catalepsy bar and paw test

A significant effect of treatment was found for the bar test ( $F_{2,21}=108.97$ ,  $P<0.0001$ ), as well as for the forelimb retraction time ( $F_{2,21}=12.02$ ,  $P<0.001$ ) and the hindlimb retraction time ( $F_{2,21}=145.80$ ,  $P<0.0001$ ) of the paw test. Post-hoc comparisons revealed that the mean scores of FAUC 213-treated animals did not differ from those of the VEH group, but haloperidol-treated animals had significantly elevated scores (all  $P<0.001$ ) (Fig. 5).



**Fig. 5** Catalepsy scores of FAUC 213 (30 mg/kg, p.o.) versus vehicle and haloperidol (0.3 mg/kg, i.p.). Values are means $\pm$ SEM of  $n=8$  rats per group. FRT/HRT indicates the time for an animal to remove one fore or hindlimb in the paw test. All measured times are scored according to a square root transformed time scale:  $t<0.09=1$ ,  $t<0.36=2$ ,  $t<0.81=3$ ,  $t<1.44=4$ ,  $t\geq1.44=5$  (\*\*\*)  $P<0.0001$ , \*\*  $P<0.001$ )

#### Effects of FAUC 213 (30 mg/kg) vs haloperidol (0.3 mg/kg) and vehicle on dopamine metabolism in mPFC, NAcc and dStr

Analysis of dopamine turnover showed a highly significant effect of treatment for the NAcc ( $F_{2,21}=12.68$ ,  $P<0.001$ ) and the dStr ( $F_{2,21}=63.72$ ,  $P<0.0001$ ), with a tendency towards significance for the mPFC ( $F_{2,21}=3.22$ ,  $P<0.065$ ). Post-hoc comparisons revealed that the ratio of dopamine turnover was significantly increased by haloperidol compared with both vehicle and FAUC 213 (NAcc:  $P<0.001$ ; dStr:  $P<0.0001$ ; mPFC:  $P<0.05$ ), whereas FAUC 213-treated animals had a dopamine turnover virtually identical to that of vehicle-treated animals (Fig. 6).

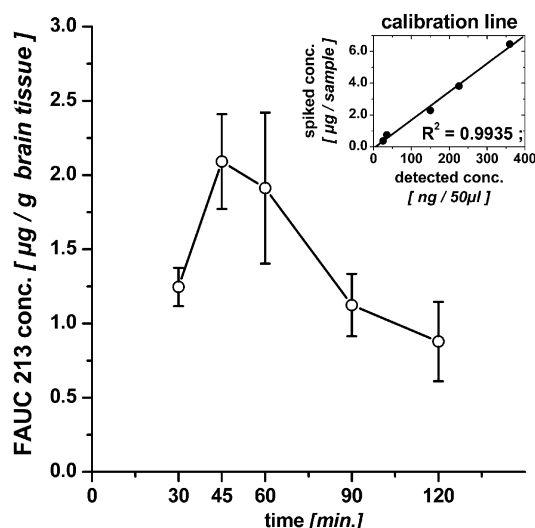
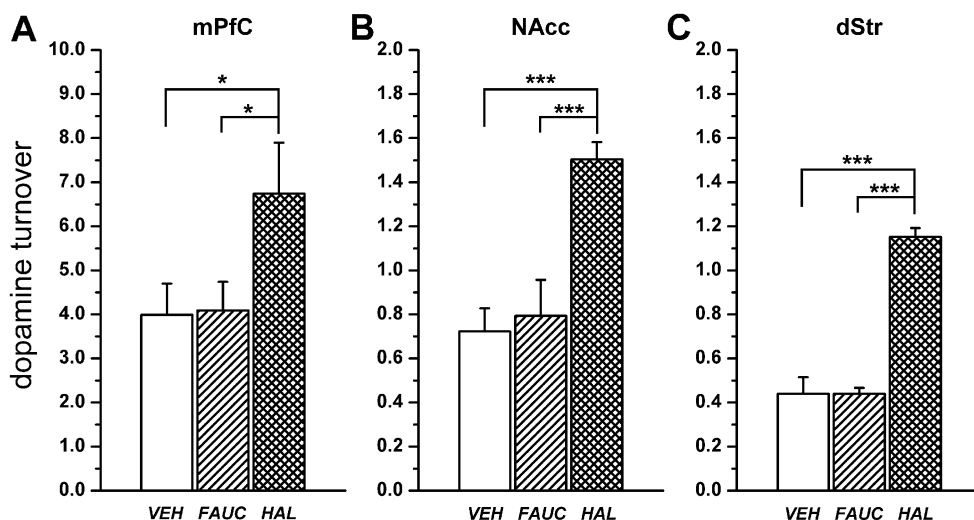
#### Pharmacokinetic profile of FAUC 213

As shown in Fig. 7, brain levels of FAUC 213 determined 30, 45, 60, 90 and 120 min after peroral application (30 mg/kg) produced a “bateman-like” kinetic curve typical for this kind of drug administration. FAUC 213 penetrated rapidly into the brain, where it reached its highest concentration ( $2.09\pm0.63$   $\mu\text{g/g}$ ) after 45 min and stayed at this level for about 15 min. This time period was in good agreement with the PPI experimental schedule. In addition, there was an interesting correlation between the decline of substance concentration in the brain after about 100 min, to less than half of  $C_{\text{max}}$ , with the convergence of the 30-mg/kg FAUC curve to the VEH+AMPH curve in amphetamine-induced locomotor activity monitoring at about the same time. The calibration curve was calculated by linear regression of the detected amount of FAUC 213 against the spiked amount and yielded  $r^2\sim0.994$ .

## Discussion

As the treatment of both positive and negative symptoms of schizophrenia, together with a necessarily good compliance achievable by the absence of adverse side effects such as extrapyramidal motoric symptoms or agranulocytosis, remains an unsolved challenge, many investigative efforts have been made to understand the basis of receptor interaction responsible for the atypical properties of clozapine. Due to the considerable affinities it shows to a number of pharmacological targets like  $D_2$ ,  $D_4$ ,  $5\text{-HT}_{2A/C}$ ,  $3_1$  and  $M_1$  (for  $D_2$ ,  $D_4$  and  $5\text{-HT}_{2A/C}$  see Table 1,  $K_i(\alpha_1)=3.7$  nM and  $K_i(M_1)=0.98$  nM as reviewed by Arnt and Skarsfeldt 1998), the value of each single receptor, or possible combination of receptors, is hard to determine. As a consequence, different working hypotheses were established following the strategy of imitating one or more elements of that binding profile to gain the same superior clinical quality without the liability of causing the rare, but severe, side effect of agranulocytosis. Some of these have focused on balanced  $5\text{-HT}_2/D_2$ -affinity often also including high  $\alpha_1$ -affinity, as shown by risperidone, or affinity

**Fig. 6** Effects of FAUC 213 (30 mg/kg, p.o.) versus haloperidol (0.3 mg/kg, i.p.) and vehicle on the levels of dopamine turnover in the medial prefrontal cortex (A), the nucleus accumbens (B) and the dorsal striatum (C). Dopamine turnover is calculated as (DOPAC + HVA)/DA. Data are expressed as means $\pm$ SEM of  $n=8$  rats per group (\*\*\* $P<0.001$ , \* $P<0.05$ )



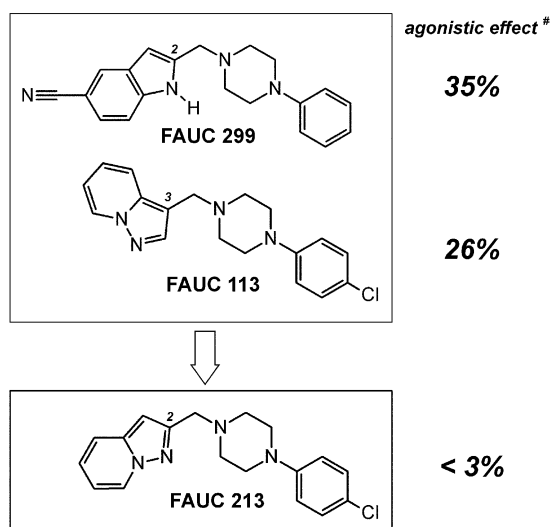
**Fig. 7** Brain levels of FAUC 213 (30 mg/kg, p.o.) at five different times after application. Animals were decapitated after 30, 45, 60, 90 and 120 min. Concentrations of FAUC 213 were determined by reversed-phase high-performance liquid chromatography analysis using ultraviolet detection after extraction from the brain matrix. The system was calibrated by spiking brain tissue of untreated animals yielding a linear regression of  $r^2 \sim 0.9935$ . The  $y$ -axis represents relative concentrations of FAUC 213 in  $\mu\text{g/g}$  wet brain tissue (weighted in frozen condition). Concentrations are presented as mean values $\pm$ SEM of  $n=3-4$  animals for each time point

for muscarinic receptors, like olanzapine (reviewed by Arnt and Skarsfeldt 1998). But, because of these multiple receptor interactions (often also including 5-HT<sub>2C</sub> or H<sub>1</sub>), these substances produce a number of other side effects such as weight gain, sedation, dizziness, postural hypotension or dry mouth (Rowley et al. 2001). Another branch of research was the evaluation of highly selective substances acting at only one target. Due to the moderate selectivity of clozapine for D<sub>4</sub> versus D<sub>2</sub> receptors and the finding that D<sub>4</sub> receptors seem to be preferentially located in limbic or cortical structures suspected of being associated with schizophrenia, whereas only very low levels are found in the striatum and other regions

modulating motor functions, the dopamine D<sub>4</sub> receptor subtype was considered to be one of the most interesting targets. With regard to this, FAUC 213 was designed by studies of regioisomers using FAUC 113 and FAUC 299 as lead structures (Löber et al. 2001) and was shown to be a complete antagonist in [<sup>3</sup>H]thymidine incorporation as evidence for mitogenic activity in contrast to its partial agonist precursors (Fig. 8). In addition, FAUC 213 had superior binding properties relative to FAUC 113, a higher affinity towards D<sub>4</sub> ( $K_i=2.2$  nM vs 3.6 nM), as well as an increased selectivity of about 2900-fold for D<sub>4.4</sub> vs D<sub>2</sub>, 240-fold for D<sub>4.4</sub> vs 5-HT<sub>2</sub> and 120-fold for D<sub>4.4</sub> vs  $\alpha_1$  (Table 1). In order to study the putative antipsychotic effects of FAUC 213, it was examined in several well-known models of behavioural neurobiology and neurochemistry, including pharmacokinetic validation of the applied parameters and doses. Throughout the whole study, male Wistar rats were used as subjects of experiment, as FAUC 213 showed binding affinities at rat-D<sub>2</sub> and D<sub>4</sub>-receptors [ $K_i(\text{rD}_2)=1.1$  nM and  $K_i(\text{rD}_4)>1000$  nM], which are in line with the binding affinities at the human D<sub>2</sub> and D<sub>4</sub> receptors (Table 1).

Stimulant-induced hypermotility, as a common model of in vivo screening of putative antipsychotics, was employed to determine effective doses of FAUC 213 when given perorally to rats. Pilot studies suggested, that adequate doses of FAUC 213 should start at about 5–10 mg/kg. We therefore chose 7.5 mg/kg as the lowest dose, and in addition used 15 mg/kg and 30 mg/kg by doubling the dose for dose-response studies. No significant effects on modification of spontaneous locomotor activity were observed at doses up to 30 mg/kg (p.o.). This served as a control that the drug itself does not exhibit unspecific, especially sedating, effects. In contrast, a dose of 30 mg/kg FAUC 213 significantly decreased AMPH-induced hyperactivity. Such an attenuation of hyperactivity is shown both by typical (like haloperidol), as well as atypical antipsychotics (such as clozapine or risperidone). It is therefore believed that direct D<sub>2</sub> antagonism may





**Fig. 8** Chemical structures of the two  $D_4$ -selective partial antagonists FAUC 113 and FAUC 299 as precursors of the  $D_4$ -selective complete antagonist FAUC 213. <sup>#</sup>Agonistic effects are given by the rate of [ $^3H$ ]-thymidine incorporation as evidence for mitogenetic activity related to the full agonistic effect of quinpirole (100%), according to Hübner et al.(2000) and Löber et al. (2001)

contribute much to this effect, although other receptors ( $D_4$ ,  $\alpha_1$ , 5-HT<sub>2A/C</sub>, etc.) undoubtedly have some impact.

PPI of acoustic startle in rats, one of the most intensively studied animal models with predictive validity for antipsychotic properties of compounds (reviewed by Geyer et al. 2001), was also used for evaluating FAUC 213. As a model of sensorimotor gating, the PPI paradigm reduces the startle reflex by a weak prepulse preceding the startle pulse, without it having the power to induce a startle reflex by itself. This phenomenon has been shown to be remarkably attenuated in schizophrenic patients. Analogically, it is disruptable in rodents or humans by treatment with, for instance, dopamine agonists (apomorphine) or dopamine releasers (amphetamine, Braff et al. 2001) and can be restored by typical and atypical antipsychotics. In accordance with the results obtained by measuring amphetamine-induced locomotor activity, we decided to perform the experiment at treatment levels of 15 mg/kg and 30 mg/kg (p.o.) FAUC 213 (the highest ineffective and the effective doses). Testing of higher doses was considered to be not appropriate, to the best of our knowledge, as we would lose receptor specificity. A dose-related increase in PPI compared with the vehicle-pretreated apomorphine group was found, with a significant effect at 30 mg/kg. Additionally, no significant effects on startle magnitude were detected, which indicates that the effects on PPI are not influenced by unspecific drug action such as sedation. These results confirm previously seen effects and also seem to concur with other investigations on predominantly  $D_4$  selective compounds (Bristow et al. 1997; Mansbach et al. 1998), which reported significant restoration of the apomorphine-induced PPI deficit only at elevated doses. This raises the question whether receptors other than  $D_4$  play a (major) role in the observed effects. Although mixed  $D_2/5\text{-HT}_2$

antagonists were reported to decrease the effect of apomorphine on PPI (reviewed by Geyer et al. 2001), the pure 5-HT<sub>2</sub> antagonist MDL 100,907 restored PPI deficits caused by 5-HT<sub>2A</sub> agonists, NMDA antagonists and isolation rearing, but not apomorphine (Geyer et al. 1999). Likewise the  $\alpha_1$  antagonist prazosine was shown to have an impact on the phencyclidine, but not apomorphine disruption of PPI (Bakshi and Geyer 1997). As a result of data obtained from knock-out mice (Dulawa et al. 2000), the activation of 5-HT<sub>1A</sub> receptors is believed to increase PPI. Therefore, the 5-HT<sub>1A</sub> antagonist (-)-UH-301 decreased PPI, whereas the 5-HT<sub>1A</sub> antagonist WAY 100,135 showed an effect on neither PPI nor on restoration of PPI disruption caused by the  $D_2/D_3$  agonist 7-OH-DPAT (reviewed by Geyer et al. 2001). All these data suggest that antagonism at neither  $\alpha_1$ , 5-HT<sub>1A</sub>, nor 5-HT<sub>2</sub> receptors is likely to contribute to the observed effects. Because  $D_2$  receptor occupation is known to be correlated to the adverse side effect EPS, we investigated whether  $D_2$  receptors are involved in the drug action at a dose level of 30 mg/kg FAUC 213. Therefore, it was tested in models of catalepsy against 0.3 mg/kg haloperidol as positive control. FAUC 213 clearly had no effect in either the bar test (Kuschinsky and Hornykiewicz 1972) or the paw test (Ellenbroek et al. 1987). As exemplified by haloperidol, typical antipsychotics usually reduce stimulated hypermotility at the same dose levels that induce catalepsy in rats, since both effects are most likely to be caused by  $D_2$  antagonism. Atypical antipsychotics, however, are devoid of cataleptic effects at doses potent in stimulated hypermotility (Arnt and Skarsfeldt 1998). The conclusion is, therefore, that FAUC 213 is not thought to be mediating its effects via a high  $D_2$  receptor occupation.

To further establish this deduction, we investigated the neurochemical effects of FAUC 213 versus haloperidol in three different brain regions known to be involved in antipsychotic drug action by postmortem HPLC analysis of dopamine and its metabolites. In both the NAcc and the dStr, haloperidol (0.3 mg/kg) produced significantly elevated levels of HVA and DOPAC, which contributed mainly to the increase of the dopamine turnover ratio, whereas FAUC 213 did not alter either DOPAC- and HVA- or DA levels. In the mPFC, haloperidol decreased DA levels, but only slightly increased levels of HVA and DOPAC, resulting in a nonetheless higher DA turnover, whereas FAUC 213 again did not alter DA levels and only slightly elevated the sum of DOPAC- and HVA levels. The results for haloperidol correspond to previous investigations (Karoum and Egan 1992; Patel et al. 1997), except for the fact that the increase in the turnover ratio in mPFC was mediated by DA decrease instead of increase of DOPAC+HVA. As FAUC 213 had no effect on dopamine turnover in NAcc and dStr, and only a negligible effect on the DOPAC+HVA levels in mPFC, it is supposed that this is due to a lack of activity at presynaptic  $D_2$  receptors and the absence of presynaptic  $D_4$  receptors. The blocking of the inhibitory effect of such presynaptic receptors on the release of dopamine into the synaptic gap followed by subsequent metabolism to HVA or DOPAC (Westerink

1985) might not be the only possible explanation for the observed results, but it seems to be more plausible and concrete than the assumption of postsynaptic receptor blockade followed by a positive feedback mechanism on this synapse or on other dopaminergic neurons in this area. The lack of effect on dopamine metabolism shown at a dose of 30 mg/kg of FAUC 213 in this study, together with the inability to cause catalepsy, may account for the fact that, in contrast to haloperidol, there are not enough D<sub>2</sub> receptors occupied in brain regions controlling motor functions in order to cause extrapyramidal side effects.

Furthermore, our pharmacokinetic investigations revealed that the nominally high dose of 30 mg/kg only results in mean C<sub>max</sub> levels of about 2.1 µg (6.4 nmol) per gram brain tissue. This might be due to weak peroral availability, efficient metabolising mechanisms or poor galenic formulation. In addition, the pharmacokinetic data confirm that the behavioural and neurochemical experiments were conducted when optimal levels of FAUC 213 were present in the brain.

## Conclusions

Peroral administration of the dopamine D<sub>4</sub> receptor full antagonist FAUC 213 yielded a significant effect in both the reduction of amphetamine-induced locomotor hyperactivity and in the restoration of apomorphine-disrupted PPI at a minimal effective dose of 30 mg/kg. Although this dose seemed to be nominally high, only a small fraction was found present in brain after a period of 45–60 min. Additionally, this dose level proved not to be high enough to induce catalepsy in rats or to increase dopamine turnover in the dStr, NAcc and mPFC by antagonism of D<sub>2</sub> receptors. In summary, we have gathered evidence, that FAUC 213 exhibits atypical antipsychotic characteristics, although it remains uncertain whether other receptors such as adrenergic or serotonergic were partially involved in the effects found in this study. The D<sub>4</sub> antagonist FAUC 213 should, therefore, be further examined.

**Acknowledgements** This work was supported by grants from the ETH Zürich, Switzerland and the Friedrich-Alexander University of Erlangen, Germany. We thank Mr. A.N.A. Ipekian for linguistic revision of the manuscript.

## References

- Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR (1997) Cellular distribution of the rat D<sub>4</sub> dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res* 752:26–34
- Arnt J, Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology* 18:63–101
- Bakshi VP, Geyer MA (1997) Phencyclidine-induced deficits in prepulse inhibition of startle are blocked by prazosin, an alpha-1 noradrenergic antagonist. *J Pharmacol Exp Ther* 283:666–674
- Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology* 156:234–258
- Bristow LJ, Collinson N, Cook GP, Curtis N, Freedman SB, Kulagowski JJ, Leeson PD, Patel S, Ragan CI, Ridgill M, Saywell KL, Tricklebank MD (1997) L-745,870, a subtype selective dopamine D<sub>4</sub> receptor antagonist, does not exhibit a neuroleptic-like profile in rodent behavioral tests. *J Pharmacol Exp Ther* 283:1256–1263
- Chabert C, Cavegn C, Bernard A, Mills A (1994) Characterisation of the functional activity of dopamine ligands at human recombinant dopamine D<sub>4</sub> receptors. *J Neurochem* 63:62–65
- Defagot MC, Malchiodi EL, Villar MJ, Antonelli MC (1997) Distribution of D<sub>4</sub> dopamine receptor in rat brain with sequence specific antibodies. *Mol Brain Res* 45:1–12
- De La Garza R II, Madras BK (2000) [(3)H]PNU-101958, a D(4) dopamine receptor probe, accumulates in prefrontal cortex and hippocampus of non-human primate brain. *Synapse* 37:232–244
- Dulawa SC, Gross C, Stark KL, Hen R, Geyer MA (2000) Knockout mice reveal opposite roles for serotonin 1A and 1B receptors in prepulse inhibition. *Neuropsychopharmacology* 22:650–659
- Ellenbroek BA, Peeters BW, Honig WM, Cools AR (1987) The paw test: a behavioural paradigm for differentiating between classical and atypical neuroleptic drugs. *Psychopharmacology* 93:343–348
- Gazi L, Bobinac I, Danzeisen M, Schupbach E, Bruinvels AT, Geisse S, Sommer B, Hoyer D, Tricklebank M, Schoeffer P (1998) The agonist activities of the putative antipsychotic agents, L-745,870 and U-101958 in HEK293 cells expressing the human dopamine D<sub>4.4</sub> receptor. *Br J Pharmacol* 124:889–896
- Gazi L, Bobinac I, Danzeisen M, Schupbach E, Langenegger D, Sommer B, Hoyer D, Tricklebank M, Schoeffer P (1999) Receptor density as a factor governing the efficacy of the dopamine D<sub>4</sub> receptor ligands, L-745,870 and U-101958 at human recombinant D<sub>4.4</sub> receptors expressed in CHO cells. *Br J Pharmacol* 128:613–620
- Geyer MA, Swerdlow NR, Mansbach RS, Braff DL (1990) Startle response models of sensorimotor gating and habituation deficits in schizophrenia. *Brain Res Bull* 25:485–498
- Geyer MA, Krebs-Thomson K, Varty GB (1999) The effects of M100907 in pharmacological and developmental animal models of prepulse inhibition deficits in schizophrenia. *Neuropsychopharmacology* 21:S134–S142
- Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* 156:117–154
- Hübner H, Kraxner J, Gmeiner P (2000) Cyanindole derivatives as highly selective dopamine D<sub>4</sub> receptor partial agonists: solid-phase synthesis, binding assays, and functional experiments. *J Med Chem* 43:4563–4569
- Karoum F, Egan MF (1992) Dopamine release and metabolism in the rat frontal cortex, nucleus accumbens, and striatum: a comparison of acute clozapine and haloperidol. *Br J Pharmacol* 105:703–707
- Koch M (1999) The neurobiology of startle. *Prog Neurobiol* 59:107–128
- Kuschinsky K, Hornykiewicz O (1972) Morphine catalepsy in the rat: relation to striatal dopamine metabolism. *Eur J Pharmacol* 19:119–122
- Lahti RA, Roberts RC, Cochrane EV, Primus RJ, Gallagher DW, Conley RR, Tamminaga CA (1998) Direct determination of dopamine D<sub>4</sub> receptors in normal and schizophrenic brain tissue: a [<sup>3</sup>H]NGD 94-1 study. *Mol Psychiatry* 3:528–533
- Löber S, Hübner H, Utz W, Gmeiner P (2001) Rationally based efficacy tuning of selective dopamine D<sub>4</sub> receptor ligands leading to the complete antagonist 2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-a]pyridine (FAUC 213). *J Med Chem* 44:2691–2694

- Mansbach RS, Brooks EW, Sanner MA, Zorn SH (1998) Selective dopamine D<sub>4</sub> receptor antagonists reverse apomorphine-induced blockade of prepulse inhibition. *Psychopharmacology* 135:194–200
- Matsumoto M, Hidaka K, Tada S, Tasaki Y, Yamaguchi T (1995) Full-length cDNA cloning and distribution of human dopamine D<sub>4</sub> receptor. *Mol Brain Res* 29:157–162
- Matsumoto M, Hidaka K, Tada S, Tasaki Y, Yamaguchi T (1996) Low levels of mRNA for dopamine D<sub>4</sub> receptor in human cerebral cortex and striatum. *J Neurochem* 66:915–919
- Mrzljak L, Bergson C, Pappy M, Huff R, Levenson R, Goldman-Rakic PS (1996) Localization of dopamine D<sub>4</sub> receptors in GABAergic neurons of the primate brain. *Nature* 381:245–248
- Mulcrone J, Kerwin RW (1997) The regional pattern of D<sub>4</sub> gene expression in human brain. *Neurosci Lett* 234:147–150
- Newman-Tancredi A, Audinot V, Chaput V, Verri  le L, Millan MJ (1997) [<sup>35</sup>S]-Guanosine-5'-O-(3-thio)triphosphate binding as a measure of efficacy at human recombinant dopamine D<sub>4</sub> receptors: actions of antiparkinsonian and antipsychotic agents. *J Pharmacol Exp Ther* 282:181–191
- Patel S, Freedman S, Chapman KL, Emms F, Fletcher AE, Knowles M, Marwood R, McAllister G, Patel S, Curtis N, Kulagowski JJ, Leeson PD, Ridgill M, Graham M, Matheson S, Rathbone D, Watt AP, Bristow LJ, Rupniak NMJ, Baskin E, Lynch JJ, Ragan CI (1997) Biological profile of L-745,870, a selective antagonist with high affinity for the dopamine D<sub>4</sub> receptor. *J Pharmacol Exp Ther* 283:636–647
- Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, New York
- Primus RJ, Thurkauf A, Xu J, Yevich E, McInerney S, Shaw K, Tallman JF, Gallagher DW (1997) II. Localisation and characterisation of dopamine D<sub>4</sub> binding sites in rat and human brain by use of the novel D<sub>4</sub> receptor selective ligand [<sup>3</sup>H]NGD 94-1. *J Pharmacol Exp Ther* 282:1020–1027
- Remington G, Kapur S (2000) Atypical antipsychotics: are some more atypical than others? *Psychopharmacology* 148:3–15
- Reynolds GP, Mason SL (1995) Absence of detectable striatal dopamine D<sub>4</sub> receptors in drug-treated schizophrenia. *Eur J Pharmacol* 281:R5–R6
- Roth BL, Tandra S, Burgess LH, Sibley DR, Meltzer HY (1995) D<sub>4</sub> dopamine receptor binding affinity does not distinguish between typical and atypical antipsychotic drugs. *Psychopharmacology* 120:365–368
- Rowley M, Bristow LJ, Hutson PH (2001) Current and novel approaches to the drug treatment of schizophrenia. *J Med Chem* 44:477–501
- Seeman P (1995) Therapeutic receptor-blocking concentrations of neuroleptics. *Int Clin Psychopharmacol* 10:5–13
- Seeman P, Van Tol HHM (1995) Deriving the therapeutic concentrations for clozapine and haloperidol: the apparent dissociation constant of a neuroleptic at the dopamine D<sub>2</sub> or D<sub>4</sub> receptor varies with the affinity of the competing radioligand. *Eur J Pharmacol* 291:59–66
- Seeman P, Guan HC, Van Tol HHM (1993) Dopamine D<sub>4</sub> receptors elevated in schizophrenia. *Nature* 365:441–445
- Seeman P, Guan H-C, Van Tol HHM (1995) Schizophrenia: elevation of dopamine D<sub>4</sub>-like sites, using [<sup>3</sup>H]nemonapride and [<sup>125</sup>I]epidepride. *Eur J Pharmacol* 286:R3–R5
- Seeman P, Corbett R, Van Tol HHM (1997) Atypical neuroleptics have low affinity for dopamine D<sub>2</sub> receptors or are selective for D<sub>4</sub> receptors. *Neuropsychopharmacology* 16:93–110
- Snyder SH (1996) Drugs and the brain. Scientific American Library, New York
- Van Tol HHM, Bunzow JR, Guan H-C, Sunahara RK, Seeman P, Niznik HB, Civelli O (1991) Cloning of the gene for a human dopamine D<sub>4</sub> receptor with high affinity for the antipsychotic clozapine. *Nature* 350:610–614
- Westerink BHC (1985) Sequence and significance of dopamine metabolism in the rat brain. *Neurochem Int* 7:221–227